Am J Trop Med Hyg, 38(2), 1988, pp 440–446 (0002-9637)

VECTOR POTENTIAL OF SELECTED NORTH AMERICAN MOSQUITO SPECIES FOR RIFT VALLEY FEVER VIRUS

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Abstract. Selected North American mosquito species were evaluated as potential vectors of Rift Valley fever virus. Field populations of *Aedes canadensis, Ae. cantator, Ae. excrucians, Ae. sollicitans, Ae. taeniorhynchus, Ae. triseriatus, Anopheles bradleyi-crucians, Culex salinarius, Cx. tarsalis,* and *Cx. territans* perorally exposed to $10^{6.2}$–$10^{7.2}$ plaque forming units of Rift Valley fever virus readily became infected. Infection rates ranged from 51% (65/127) for *Cx. salinarius* to 96% (64/67) for *Ae. canadensis.* Disseminated infection rates were generally greater at 14 days than at 7 days after the infectious bloodmeal, and, with the exception of *An. bradleyi-crucians,* they were not significantly different than the pooled rate of 59% for each species tested. Only 5/55 (9%) of the *An. bradleyi-crucians* developed a disseminated infection. For most of the species, about half of the mosquitoes with a disseminated infection transmitted an infectious dose of virus to hamsters. While all species, with the exception of *An. bradleyi-crucians,* transmitted virus, *Ae. canadensis, Ae. taeniorhynchus,* and *Cx. tarsalis* had the highest vector potential of the species tested. Following inoculation of approximately $10^{7.6}$ plaque forming units of virus, 100% of the mosquitoes of each species became infected. For most species, transmission rates were similar for inoculated individuals and those that developed a disseminated infection following peroral infection. Viral titers of transmitting and nontransmitting-disseminated individuals were similar for all species tested. These data suggest that, if Rift Valley fever virus was introduced into North America, several mosquito species would be capable of transmitting it.

Rift Valley fever (RVF) was first described as a veterinary disease of domesticated ruminants in Kenya in 1931.[1] This viral infection is now recognized as a serious, sometimes fatal disease of humans. Potential exportation of RVF virus from the enzootic sub-Saharan region of Africa has been a concern for many years.[2–4] In 1977, RVF virus was introduced into the Nile Delta of Egypt and resulted in extensive morbidity and mortality among humans and livestock.[5–7] A subsequent report of a Canadian woman who experienced a febrile illness of 2–3 days duration while on safari in Mombasa, Kenya, provided a dramatic example of potential RVF virus introduction into new geographic areas, including North America.[8] These events reinforce Easterday's[9] observations that ". . . RVF is a potential threat to the livestock industry of the United States and many other countries . . ." and that, "the possibility of the virus being introduced in infected human beings . . . must also be considered."

Because of the potential importation of RVF virus into North America, we initiated a study to evaluate the vector potential of selected mosquito species. Selection of the species tested was generally based on one or more of the following criteria: those that are known to feed on both large mammals and humans; those that occur in large numbers; and those that have been incriminated as vectors of other arboviruses.

MATERIALS AND METHODS

The following definitions are offered for clarification. *Infection rate* is the proportion of mosquitoes that contain RVF virus 7 or more days following exposure to the virus. *Dissemination rate* is the proportion of infected mosquitoes with virus in their legs. The *transmission per disseminated rate* (T_D) is the proportion of mosquitoes with a disseminated viral infection that transmitted virus to a susceptible host. The *transmission rate* is the proportion of all mosquitoes (infected and uninfected) that transmitted virus to a susceptible host. The *transmission per in-*

Accepted 6 August 1987.

TABLE 1

North American mosquito species evaluated for vector potential of Rift Valley fever virus

Species	Collection data	
	Stage	Locality
Aedes (*Aedes*)		
cinereus Meigen	Larva	Gogebic Co., MI
Aedes (*Ochlerotatus*)		
abserratus (Felt & Young)	Larva	Gogebic Co., MI
canadensis (Theobald)	Adult	Worcester Co., MD
cantator (Coquillett)	Adult	Somerset Co., MD
diantaeus Howard	Larva	Gogebic Co., MI
excrucians (Walker)	Larva	Gogebic Co., MI
fitchii (Felt & Young)	Larva	Gogebic Co., MI
sollicitans (Walker)	Adult	Accomack Co., VA
taeniorhynchus (Wiedemann)	Adult	Accomack Co., VA
Aedes (*Protomacleaya*)		
triseriatus (Say)	Egg	Worcester Co., MD
Anopheles (*Anopheles*)		
bradleyi King-*crucians* Wiedemann	Adult	Somerset Co., MD
Culex (*Culex*)		
salinarius Coquillett	Adult	Somerset Co., MD
tarsalis Coquillett	Larva	Kern Co., CA
Culex (*Neoculex*)		
territans Walker	Adult	Somerset Co., MD

oculated rate (T_i) is the proportion of virus-inoculated mosquitoes that transmitted virus to a susceptible host.

Mosquito species

A previous study with an Egyptian strain of *Culex pipiens* and RVF virus demonstrated the potential adverse effects of laboratory colonization on vector potential.[10] Therefore, all mosquitoes used in this study were obtained from the field as either eggs, larvae, or adults (Table 1).

Adult mosquitoes were collected with miniature light traps supplemented with CO_2 (0.5 kg dry ice), and larvae were collected with dippers. Adults were maintained on apple slices and a 5% sucrose solution in either 30.5 cm^3 screen cages or 3.8 liter cardboard containers with screen tops in bioclimatic chambers at 26°C, 70%–80% RH, and a 15 hr photophase. *Cx. tarsalis* larvae were reared to adults in California and shipped to USAMRIID for testing. Larve collected in Michigan from pools formed by the melting of snow and ice were reared in bioclimatic chambers at 18°C until adult eclosion and maintained as adults at 26°C. Other details of the mosquito rearing and handling techniques were described previously.[10]

Virus and viral assays

The ZH501 strain of RVF virus was isolated during an epizootic in Egypt and passaged twice in fetal rhesus monkey lung cells.[11] Specimens were triturated as described previously stored at −70°C until assayed for virus by plaque assay in Vero cells.[12] Mosquito legs and bodies were assayed separately to determine the viral dissemination status of individual mosquitoes.

Infection of mosquitoes

Mosquitoes were exposed to RVF virus either by intrathoracic inoculation[14] with approximately $10^{1.6}$ plaque forming units (PFU) of virus or by allowing them to feed on anesthetized golden Syrian hamsters (*Mesocricetus auratus*) that had been inoculated intraperitoneally 24 hr earlier with $10^{4.0}$ PFU of RVF virus. The amount of virus ingested by or inoculated into mosquitoes was determined by assaying 3–5 mosquitoes individually from each group immediately following virus exposure. After per os exposure (≈2

TABLE 2

*Rift Valley fever virus infection, dissemination, and transmission rates for mosquitoes that ingested a high dose of virus**

Species	Incubation day	Infection†	Dissemination‡	Transmission per disseminated (T_D)§	Transmission¶
Aedes					
canadensis	7	—**	19 37 (51)	4 19 (21)	4 39 (10)
	14	64 67 (96)	12 13 (92)	7 12 (58)	7 13 (54)
cantator	7, 14	46 54 (85)	17 27 (63)	1 17 (6)	1 35 (3)
excrucians	7, 14	28 39 (71)	1 5 (20)	1 1 (100)	1 9 (11)
sollicitans	7, 14	123 137 (90)	56 82 (68)	16 56 (29)	16 92 (17)
taeniorhynchus	7	—	—	4 17 (24)	4 33 (12)
	14	165 193 (85)	43 63 (68)	15 26 (58)	15 36 (42)
triseriatus	7	—	—	5 9 (56)	5 14 (36)
	14	71 86 (83)	18 27 (67)	0 9 (0)	0 15 (0)
Anopheles					
bradleyi-crucians	7, 14	74 89 (83)	5 55 (9)	0 5 (0)	0 69 (0)
Culex					
salinarius	7	75 105 (71)	12 48 (25)	—	1 66 (2)
	14	65 127 (51)	25 32 (78)	7 37 (19)	6 54 (11)
tarsalis	7, 14	45 52 (88)	5 10 (50)	4 5 (80)	4 11 (36)
territans	7	—	0 9 (0)	NA	0 34 (0)
	14	39 53 (74)	5 7 (71)	2 5 (40)	2 8 (25)

* Mean = $10^{8.3}$ PFU, n = 124, range $10^{7.3}$–$10^{9.3}$ PFU.
† No. infected/No. tested (% positive).
‡ No. with virus-infected leg samples that refed/No. infected that refed (% disseminated).
§ No. disseminated that transmitted/No. disseminated that refed (% T_D).
¶ No. that transmitted/total No. that refed (% transmission).
** When the rates for days 7 and 14 were not significantly different from each other, they were pooled and listed under day 14. If they were significantly different by the χ^2 test ($P = 0.05$) then they were listed separately for days 7 and 14.
NA = not applicable.

hr), the mosquitoes were anesthetized with CO_2 and replete females were removed and transferred to a new cage and maintained as described above. Partially engorged and unengorged females were discarded. To monitor viral replication, dissemination, and the ability of mosquitoes to transmit virus, samples were tested after different incubation periods and then stored at −70°C until assayed for virus.

Viral transmission

Four days after per os exposure to RVF virus, mosquitoes were provided an appropriate oviposition substrate. These mosquitoes were allowed to refeed individually on susceptible hamsters following 7 and 14 days incubation.[10] Mosquitoes were placed individually in either large screen cardboard cartons (3.8 liter) to which a hamster was added or in small screen cages (6 × 3 × 2 cm) which were then placed individually on the abdomens of anesthetized hamsters. Hamsters were then caged individually and observed for a minimum of 21 days. Because infection with RVF virus is essentially 100% fatal for hamsters,[10] hamster death was used as the criterion for virus transmission. Approximately 4 hr after each transmission attempt, species identification was confirmed and feeding status determined under a dissecting microscope. All mosquitoes were then frozen at −70°C until assayed for virus. Mosquitoes inoculated with virus were allowed to refeed individually on susceptible hamsters after incubation for 10–12 days, and thereafter handled as the per os exposed mosquitoes.

RESULTS

Oral exposure

All species tested were readily susceptible to infection with RVF virus following ingestion of between $10^{6.3}$ and $10^{9.3}$ PFU of this virus (Table 2). As expected, infection rates were generally lower when smaller doses of virus were ingested (data not shown). However, even when only $10^{4.1}$ PFU were ingested, infection rates were over 70% in 3 of the 4 species tested.

In contrast to the similarity of infection rates

TABLE 3

*Rift Valley fever virus transmission rates and viral titers for mosquitoes that were intrathoracically inoculated with virus**

Species	Transmission rate†	T_{50}‡	Significance§
Aedes			
abserratus	1/2 (50)	—	—
canadensis	26/26 (100)	11/31 (35)	$P < 0.001$
cantator	7/30 (23)	1/17 (6)	NS
cinereus	2/8 (25)	—	—
diantaeus	9/9 (100)	—	—
excrucians	6/21 (29)	1/1 (100)	NS
hirchi	1/16 (6)	—	—
sollicitans	41/80 (51)	22/78 (28)	$P < 0.00$
taeniorhynchus	10/15 (67)	20/47 (43)	NS
triseriatus	3/15 (20)	5/18 (28)	NS
Anopheles			
bradleyi-crucians	0/55 (0)	0/6 (0)	NS
Culex			
salinarius	52/99 (52)	8/40 (20)	$P < 0.00$
tarsalis	22/22 (100)	6/7 (86)	NS
territans	21/47 (45)	5/13 (38)	NS

* Mean $10^{x.x}$ PFU

† Number that transmitted/No. inoculated (% transmitting)

‡ Number disseminated that transmitted/No. disseminated that ruled (% transmitting)

§ Probability that the difference between the transmission rate and the T_{50} occurred by chance; NS = $P > 0.10$

among the species tested, viral dissemination to the hemocoel was significantly lower (χ^2, $P < 0.001$) in *An. bradleyi-cruciens* (5/55, 9%) than in the *Aedes* (166/254, 65%) or *Culex* (47/106, 44%) species tested (Table 2). The dissemination rates for the latter 2 genera were also significantly different (χ^2, $P < 0.001$). However, this latter difference may be accounted for by the time of extrinsic incubation when they were sampled. For instance, dissemination rates on day 14 were nearly identical, 73% and 72% for the 2 genera, respectively. In all 3 genera, dissemination rates were generally higher in mosquitoes tested on day 14 than in those tested on day 7 (data not shown). While sample sizes for most species were too small to have much statistical power, dissemination rates were significantly higher at day 14 than at day 7 for both the pooled *Aedes* species (χ^2, $P < 0.05$) and the pooled *Culex* species (χ^2, $P < 0.001$).

As with dissemination rates, transmission rates were generally higher in mosquitoes tested on day 14 than in those tested on day 7. While none of a total of 69 orally exposed *An. bradleyi-cruciens*, including 5 with disseminated infection, transmitted virus, all other species tested successfully transmitted virus by bite. However, even for mosquitoes with a disseminated infection, transmission rates were less than 100% for each species tested, with the exception of *Ae. canadensis*. The 100% T_{50} for this species was based on a single specimen.

For each of the 8 species in which there were both transmitting and nontransmitting-disseminated individuals, there was no significant difference in leg titers between the transmitting and nontransmitting-disseminated mosquitoes. Hence, leg titer could not be used to separate transmitting from nontransmitting individuals in any of the species tested.

Inoculated mosquitoes

Following inoculation with $\approx 10^{7.0}$ PFU of RVF virus, all of the mosquitoes in each species became infected. Transmission rates for orally exposed mosquitoes with a disseminated infection (including those with a disseminated infection following ingestion of $\geq 10^{5.0}$ PFU of virus) and those inoculated with RVF virus were not statistically different for most species tested (Table 3). However, inoculated *Ae. canadensis*, *Ae. sollicitans*, and *Cx. salinarius* were significantly more efficient transmitters than orally exposed individuals of these same species with disseminated infections. Not only was *An. bradleyi-cruciens* the only species that failed to transmit RVF virus (0/55), but also, the mean viral titer recovered

TABLE 4

Summary of vector potential of 9 species of North American mosquitoes that ingested Rift Valley fever virus

Species	Potential* for			Vector potential
	Infection	Dissemination	Transmission	
Aedes (Ochlerotatus)				
canadensis	+ + +	+ + +	+ +	Very good
taeniorhynchus	+ + +	+ + +	+ +	Very good
Culex (Culex) tarsalis	+ + +	+ + +	+ +	Very good
Ae. (Protomacleaya)				
triseriatus	+ + +	+ + +	+	Moderate
Cx. (Neoculex) territans	+ + +	+ + +	+	Moderate
Ae. (Och.) sollicitans	+ + +	+ + +	+	Moderate
cantator	+ + +	+ + +	+	Moderate
Cx. (Cul.) salinarius	+ + +	+ + +	+	Moderate
Anopheles (Anopheles)				
bradleyi-crucians	+ + +	+	0	Very poor

* High = + + +, medium = + +, low = +, and very poor = 0.

from this species ($10^{4.1}$ PFU/mosquito) was significantly lower ($P < 0.001$) than that recovered from any of the other species tested. However, as with the disseminated mosquitoes in the oral exposure experiment, there was no significant difference in titer between transmitting and nontransmitting inoculated mosquitoes for each species tested. Mean viral titers were similar for the *Aedes* and *Culex* species tested, and ranged from $10^{4.8}$–$10^{5.6}$ and from $10^{5.0}$–$10^{5.3}$ for the 2 genera, respectively.

DISCUSSION

These studies suggest that if RVF virus were introduced into North America, several mosquito species known to commonly feed on large mammals and humans would be capable of transmitting this virus. A relative classification of the vector potential of the per os exposed mosquitoes is presented in Table 4. The vector potential ranged from very good for *Ae. canadensis*, *Ae. taeniorhynchus*, and *Cx. tarsalis* to very poor for *An. bradleyi-crucians*. Intermediate levels of vector potential were recorded for the other *Culex* species (*salinarius* and *territans*) and *Aedes* species (*cantator*, *sollicitans*, and *triseriatus*).

There appear to be at least 3 general types of transmission patterns. In the first, represented by *An. bradleyi-crucians*, viral transmission was extremely inefficient. While this species was readily susceptible to infection with RVF virus following per os exposure, most of the *An. bradleyi-crucians* failed to develop a disseminated infection. Furthermore, when the midgut was circumvented by inoculation, this species still failed to transmit virus, suggesting the presence of a salivary gland "barrier."[15] Similar inefficient transmission has also been reported for RVF virus-inoculated *An. albimanus* (1/28, 4%) and *An. stephensi* (0/21, 0%).[16]

In the second pattern, typified by *Cx. tarsalis*, dissemination from the midgut appeared to be the primary determinant of vector competence, as nearly all of the individuals with a disseminated infection (28/29, 97%) transmitted virus. This is the same pattern reported for *Cx. pipiens*.[13,16]

A third pattern is illustrated by *Ae. taeniorhynchus*, in which both dissemination from the midgut and a "salivary gland barrier" appeared to determine vector competence. In this group, dissemination of virus from the midgut was time-dependent, with increased dissemination rates occurring with longer extrinsic incubation. However, unlike with the second pattern, dissemination from the midgut was not the sole determinant for transmission, as not all of the inoculated *Ae. taeniorhynchus* transmitted virus and only 30/62 (48%) of the individuals with a disseminated infection transmitted virus by bite.

The snow melt mosquitoes were difficult to maintain and were reluctant to feed on hamsters. Thus, their vector potential for RVF virus could not be assessed following peroral exposure. However, following inoculation, all these species

transmitted RVF virus. Thus, these species might also be competent vectors following oral exposure.

In nature, efficiency of transmission of viral agents by arthropod vectors is dependent upon many factors in addition to whether or not an arthropod can transmit in the laboratory. These include specific interactions between the vector, the pathogen, the host, and the environment, such as vector and susceptible host density, geographic distribution, longevity, dispersal patterns, and feeding preferences. If enough of these critical factors come together in space and time then it is possible for a marginal or poor laboratory vector to be transformed into a major epizootic vector in nature. For example, *Ae. sollicitans* and *Cx. salinarius* were not as efficient at transmitting RVF virus in the laboratory as was *Cx. territans*. However, because *Ae. sollicitans* and *Cx. salinarius* feed on both large mammals and humans while *Cx. territans* primarily feeds on reptiles and birds, *Ae. sollicitans* and *Cx. salinarius* probably have greater potential to serve as vectors of RVF virus in North America than does *Cx. territans*.

Mosquitoes ingested $10^{4.1}$–$10^{7.2}$ PFU of RVF virus in these studies. These titers represent viremias of $10^{6.6}$–$10^{9.7}$ PFU per ml of blood. During the RVF outbreak in Egypt, viremias in humans reached $10^{8.6}$ suckling mouse intracerebral LD_{50} (about 10^{8} PFU) per ml of blood. As the incubation period for humans is 2 to 6 days and viremias last 3 to 4 days,[17] people exposed to RVF virus in Africa could, on their return to North America, be circulating sufficient virus to infect North American mosquitoes. While there are no field data on viremia titers produced in North American strains of sheep and cattle, laboratory studies indicate that newborn sheep produce viremias in excess of 10^{9} PFU per ml of blood (M. J. Turell and C. L. Bailey, personal communication).

Hoch et al.[18] demonstrated that several North American insects could mechanically transmit RVF virus under laboratory conditions either from hamster to hamster or from hamster to sheep. Thus, in addition to the potential for biological transmission of RVF virus described in this study, there is also the potential for mechanical transmission by hematophagous insects.

Therefore, there is reason to believe that if RVF virus were introduced into North America, it might cause major epizootics in both the sheep and cattle populations and that there is a high probability that human infections would also occur.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of George B. Craig, Jr., in collecting the snow melt pool mosquitoes and William K. Reisen for supplying the *Cx. tarsalis*. We are also grateful to Ralph Tammariello for assaying specimens by the ELISA, Dwayne D. Oland for computer programming and system analysis support, and Paul Gibbs for guidance in the statistical analysis. We acknowledge the editorial assistance of Katheryn Kenyon and Kenneth Linthicum and Douglas M. Watts.

The views of the authors do not purport to reflect the positions of the U.S. Department of the Army or the U.S. Department of Defense.

REFERENCES

1. Daubney, R., Hudson, J. R., and Graham, P. C., 1931. Enzootic hepatitis or Rift Valley fever. An undescribed disease of sheep, cattle and man in East Africa. *J. Pathol. Bacteriol., 34:* 545–579.
2. Bres, P., 1981. Prevention of the spread of Rift Valley fever from the African continent. *Contrib. Epidemiol. Biostat., 3:* 178–190.
3. Lupton, H. W., Peters, C. J., and Eddy, G. A., 1982. Rift Valley fever: Global spread or global control? Pages 261–275 in *Proc. 86th Ann. Meeting U.S. Animal Health Assoc.*, Nashville, Tennessee.
4. Shope, R. E., Peters, C. J., and Davies, F. G., 1982. The spread of Rift Valley fever and approaches to its control. *Bull. WHO, 60:* 299–304.
5. Meegan, J. M., 1979. The Rift Valley fever epizootic in Egypt 1977–78. 1. Description of the epizootic and virological studies. *Trans. R. Soc. Trop. Med. Hyg., 73:* 618–623.
6. Hoogstraal, H. J., Meegan, J. M., Khalil, G. M., and Adham, F. K., 1979. The Rift Valley fever epizootic in Egypt 1977–78. 2. Ecological and entomological studies. *Trans. R. Soc. Trop. Med. Hyg., 73:* 624–629.
7. Laughlin, L. W., Meegan, J. M., Strausbaugh, L. F., Morens, D. M., and Watten, R. H., 1979. Epidemic Rift Valley fever in Egypt: Observations of the spectrum of human illness. *Trans. R. Soc. Trop. Med. Hyg., 73:* 630–633.
8. Centers for Disease Control, 1979. Rift Valley

fever with retinopathy—Canada. *Morbid. Mortal. Wkly. Rep., 28:* 607–608.

9. Easterday, B. C., 1965. Rift Valley fever. *Adv. Vet. Sci., 10:* 65–127.
10. Gargan II, T. P., Bailey, C. L., Higbee, G. A., Gad, A., and El Said, S., 1983. The effects of laboratory colonization on the vector-pathogen interactions of Egyptian *Culex pipiens* and Rift Valley fever virus. *Am. J. Trop. Med. Hyg., 32:* 1154–1163.
11. Peters, C. J., and Anderson, G. W., Jr., 1981. Pathogenesis of Rift Valley fever. *Contrib. Epidemiol. Biostat., 3:* 21–41.
12. Earley, E., Peralta, P. H., and Johnson, K. M., 1967. A plaque neutralization method for arboviruses. *Proc. Soc. Exp. Biol. Med., 125:* 741–747.
13. Turell, M. J., Gargan II, T. P., and Bailey, C. L., 1984. Replication and dissemination of Rift Valley fever virus in *Culex pipiens*. *Am. J. Trop. Med. Hyg., 33:* 176–181.
14. Rosen, L., and Gubler, D., 1974. The use of mosquitoes to detect and propagate dengue viruses. *Am. J. Trop. Med. Hyg., 23:* 1153–1160.
15. Kramer, L. D., Hardy, J. L., Presser, S. B., and Houk, E. J., 1981. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. *Am. J. Trop. Med. Hyg., 30:* 190–197.
16. Turell, M. J., and Bailey, C. L., 1987. Transmission studies in mosquitoes (Diptera: Culicidae) with disseminated Rift Valley fever virus infections. *J. Med. Entomol., 24:* 11–18.
17. Peters, C. J., and Meegan, J. M., 1981. Rift Valley fever. Pages 403–420 *in* G. W. Beran, ed., *Handbook Series in Zoonoses, Section B: Viral Zoonoses, Volume I.* CRC Press, Inc., Boca Raton, Florida.
18. Hoch, A. L., Gargan II, T. P., and Bailey, C. L., 1985. Mechanical transmission of Rift Valley fever virus by hematophagous Diptera. *Am. J. Trop. Med. Hyg., 34:* 188–193.